# Activation of Fatty Acid Synthesis in Cell-free Extracts of *Saccharomyces cerevisiae*

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Fatty acid synthesis from acetate in extracts of *Saccharomyces cerevisiae* strain LK2G12 was shown to be stimulated by α-glycerophosphate and citrate, and by a number of compounds related to them. Magnesium was shown to stimulate fatty acid synthesis from acetyl-coenzyme A but not from malonyl-coenzyme A, thus indicating the site of stimulation of fatty acid synthesis to be the acetyl-coenzyme A step.

Tricarboxylic acid cycle intermediates are known to stimulate fatty acid synthesis in extracts of animal tissues (2, 3, 7, 15). In addition, glycolytic intermediates, including α-glycerophosphate, have been shown to stimulate fatty acid synthesis in such preparations (1, 9). In some of these systems, acetyl-coenzyme A (CoA) carboxylase was shown to be the rate-limiting enzyme of fatty acid synthesis (6, 18), and tricarboxylic acid cycle intermediates were found to increase the rate of acetyl-CoA carboxylation (7, 8, 14, 16). Most of the work on this effect has been concerned with extracts of animal origin, but recent reports from this laboratory have indicated that tricarboxylic acid cycle intermediates and intermediates of the glycolytic pathway, especially α-glycerophosphate, stimulate fatty acid synthesis in extracts of *Saccharomyces cerevisiae* (19, 20). The results reported here confirm and extend these findings.

## MATERIALS AND METHODS

Information on the organism used in this study, *S. cerevisiae* strain LK2G12, its cultivation, harvesting, disruption, and subsequent centrifugal fractionation is given in earlier publications (11, 19, 20).

Lipid synthesis was followed by the incorporation of radioactive precursors. All incubations were carried out at 30 C. The incubation mixtures were saponified in alcoholic potassium hydroxide and extracted with petroleum ether as previously described (19).

Radioactivity was determined with a Packard Tri-Carb liquid scintillation counter. The scintillation fluid consisted of 1 liter of toluene, 1 liter of dioxane, 0.6 liter of 100% ethyl alcohol, 130 g of naphthalene, 13 g of diphenyloxazole, and 0.25 g of methylphenyloxazolylbenzene.

Proteins were determined by the biuret (12) or Lowry (13) method, with Prosol (bovine serum albumin) as the standard.

Reduced nicotinamide adenine dinucleotide (NADPH₂), CoA, reduced glutathione, adenosine triphosphate (ATP), and creatine kinase were purchased from the Boehringer-Mannheim Corp. (New York, N.Y.). Creatine phosphate, glycolytic intermediates, and tricarboxylic acid cycle intermediates were purchased from Sigma Chemical Co. (St. Louis, Mo.). Radioactive precursors were purchased from New England Nuclear Corp. (Boston, Mass.).

## RESULTS

*Effect of α-glycerophosphate and citrate on fatty acid synthesis.* White and Klein (20) reported that α-glycerophosphate and citrate stimulated fatty acid synthesis in high-speed supernatant preparations of yeast. We have confirmed these findings and, in addition, have found that the particulate fraction (obtained from postmitochondrial supernatant fluids, and hereafter referred to as the crude ribosomal fraction) which is active in de novo synthesis of fatty acids from acetate (10, 11) can also be stimulated by α-glycerophosphate and citrate (Table 1).

*Effect of other compounds on fatty acid synthesis.* A number of compounds metabolically or chemically related to α-glycerophosphate or citrate were tested for their effects on fatty acid synthesis. The results are compiled in Table 2, from which it can be seen that α-glycerophosphate, fructose-1,6-diphosphate, β-glycerophosphate, 6-phosphogluconate, fructose-6-phosphate, citrate, isocitrate, and adipate were highly stimulatory at all concentrations tested.

*Effects of magnesium and manganese.* Since fatty acid synthesis from acetate requires ATP, it was of interest to test the effects of magnesium and manganese, which are known to be involved in ATP-requiring reactions. The degree of response of fatty acid synthesis from acetate to

TABLE 1. *Effect of* DL-α-*glycerophosphate and citrate on fatty acid synthesis by crude ribosomal preparation*

| Additions[a] | Concn (M) | Acetate incorporation (mμmoles) |
|---|---|---|
| α-Glycerophosphate | 1 × 10⁻³ | 8.4 |
| | 1 × 10⁻³ | 15.8 |
| | 2 × 10⁻³ | 15.2 |
| | 10 × 10⁻³ | 20.8 |
| | 30 × 10⁻³ | 25.4 |
| | 40 × 10⁻³ | 44.7 |
| Citrate | 0 | 33.8 |
| | 2.5 × 10⁻³ | 46.1 |
| | 5 × 10⁻³ | 61.6 |
| | 15 × 10⁻³ | 97.4 |
| | 40 × 10⁻³ | 117.7 |

[a] Each sample contained, in a final volume of 1.0 ml, the following: 70 μmoles of potassium phosphate buffer ($pH$ 6.3), 2.4 μmoles of NADPH₂, 0.05 μmole of coenzyme-A, 2 μmoles of MnCl₂, 10 μmoles of creatine phosphate, 4.5 units of creatine kinase, 2 μmoles of ATP, 2 μmoles of acetate-*1*-¹⁴C (2 × 10⁶ counts per min per μmole), and 60 μmoles of KHCO₃. The α-glycerophosphate samples contained the amounts of α-glycerophosphate listed and dialyzed crude ribosomal preparation containing 4.10 mg of protein. The citrate samples contained the amounts of citrate listed and dialyzed crude ribosomal preparation containing 2.90 mg of protein. Samples were incubated for 20 min at 30 C.

manganese and magnesium was similar. The addition of manganese caused a 13-fold stimulation at $3 \times 10^{-3}$ M, whereas magnesium caused this amount of stimulation at about $15 \times 10^{-3}$ M. However, since the first enzyme involved in the synthesis of fatty acids from acetate, acetyl-CoA synthetase, is known to be inhibited by magnesium (5), it seemed possible that the full potential of magnesium was obscured in testing fatty acid synthesis from acetate; therefore, the effects of the two metals were tested on fatty acid synthesis from acetyl-CoA. The results in Table 3 show that magnesium caused much higher levels of stimulation than did manganese. To ascertain whether the magnesium effect was localized at the acetyl-CoA carboxylase step rather than at the fatty acid synthetase step, the effect of magnesium on the incorporation of malonyl-CoA was also studied. As can be seen from Table 4, neither manganese nor magnesium enhanced the synthesis of fatty acids from malonyl-CoA. In fact, they inhibited the incorporation.

## DISCUSSION

The large number of compounds which activate fatty acid synthesis in extracts of this strain of *S. cerevisiae* indicates that fatty acid synthesis in cell-free yeast preparations is subject to regulation in a manner quite analogous to that reported for animal extracts (2, 3, 7, 15). Of these compounds, α-glycerophosphate and fructose-1,6-diphosphate were very effective, although related compounds were also active. Since the extracts used for fatty acid synthesis were crude preparations, a number of enzymes, particularly glycolytic enzymes, undoubtedly were present. One cannot rule out the possibility, therefore, that there is only one primary activating compound among these related substances (e.g., α-glycerophosphate), and that related compounds are first metabolized to it.

It is interesting that, of the compounds related to citrate, none stimulated as well as citrate. Because the high-speed supernatants used for these experiments contained no mitochondria, and were therefore deficient in Krebs cycle enzymes, these compounds probably were not metabolized to citrate. In this connection, the stimulation by *trans*-aconitate is to be noted. Since this compound is an inhibitor of the Krebs cycle, its activating action on fatty acid synthesis cannot be due to the generation of Krebs cycle intermediates, but must be due to a property of the acid itself.

Although malonate has been reported to be a strong stimulator of fatty acid synthesis in rat liver and rat mammary gland extracts (3, 4), it did not stimulate fatty acid synthesis in these extracts. In fact, it appeared to be somewhat inhibitory.

In animal systems it has been shown that acetyl-CoA carboxylation is the rate-limiting step in fatty acid synthesis (6, 18). It has also been shown in several animal systems that tricarboxylic acid cycle intermediates increase the rate of acetyl-CoA carboxylation (7, 8, 14, 16). Similarly, we (19) have presented evidence that acetyl-CoA carboxylase is the rate-limiting step in fatty acid synthesis in extracts of *S. cerevisiae*, and that citrate, fructose-1,6-diphosphate, or α-glycerophosphate stimulate the activity of this enzyme. The experiments with magnesium salts detailed above also indicate that fatty acid synthesis is stimulated in extracts of this yeast at the carboxylation step, since magnesium caused a much greater stimulation of fatty acid synthesis from acetyl-CoA than from acetate, and caused no stimulation from malonyl-CoA.

The stimulation by α-glycerophosphate and citrate of fatty acid synthesis in the crude ribo-

TABLE 2. *Relative responses of compounds tested for effect on fatty acid synthesis*[a]

| Compounds tested | Concentration of compound tested (M) | | | |
|---|---|---|---|---|
| | $8 \times 10^{-3}$ | $20 \times 10^{-3}$ | $48 \times 10^{-3}$ | $60 \times 10^{-3}$ |
| DL-α-Glycerophosphate | 1.8 | 2.1 | 2.9 | — |
| L-α-Glycerophosphate | 1.8 | 2.0 | 2.1 | — |
| Fructose-1,6-diphosphate | 1.9 | 2.2 | 3.0 | — |
| β-Glycerophosphate | 1.5 | 1.6 | 2.4 | — |
| DL-Glyceraldehyde | 1.2 | 2.0 | 1.4 | — |
| D-Glyceraldehyde | 0.9 | 1.1 | 1.1 | — |
| DL-Glyceraldehyde-3-phosphate | 1.0 | 1.7 | 1.2 | — |
| Carbamyl phosphate | 1.1 | 1.4 | 1.6 | — |
| 6-Phosphogluconate | 1.7 | 1.3 | 1.8 | — |
| Fructose-6-phosphate | 1.8 | 1.4 | 1.6 | — |
| Fructose-1-phosphate | 1.2 | 1.1 | 1.7 | — |
| Glycerol-1,3-diphosphate | 0.9 | 1.4 | 1.2 | — |
| Glycerol-1,2-diphosphate | 1.2 | 1.6 | 1.1 | — |
| Dihydroxyacetone | 1.0 | 1.0 | 1.0 | — |
| Phosphoenolpyruvate | — | 1.0[b] | — | 1.2 |
| 3-Phosphoglycerate | — | 1.2[b] | — | 1.0 |
| 2-Phosphoglycerate | — | 1.3[b] | — | 1.4 |
| DL-Glycerate | 1.2 | 1.0 | 0.4 | — |
| Formate | — | 1.2[b] | — | 1.2[b] |
| Acetyl-phosphate | 1.0 | 0.6 | 0.6 | — |
| Glycerol | — | 1.1[b] | — | 1.0[b] |
| Pyruvate | — | 0.6[b] | — | 0.6 |
| Citrate | 1.4 | 1.9 | 2.8 | — |
| Isocitrate | 1.7 | 1.7 | 2.1 | — |
| Adipate | 1.5 | 1.9 | 2.2 | — |
| Glutarate | 1.1 | 1.4 | 2.1 | — |
| DL-Glutamate | 1.3 | 1.2 | 1.8 | — |
| DL-Lactate | — | 1.5[b] | — | 1.6 |
| D-(−)-Lactate | 1.2 | 1.4 | 1.5 | — |
| L-(+)-Lactate | 1.1 | 1.5 | 1.8 | — |
| Tricarballyate | 1.1 | 1.0 | 1.7 | — |
| Fumarate | 1.2 | 1.3 | 1.4 | — |
| α-Ketoglutarate | 1.1 | 1.3 | 1.4 | — |
| α-Ketobutyrate | 0.9 | 1.1 | 1.0 | — |
| *trans*-Aconitate | 0.9 | 1.4 | 1.4 | — |
| Malate | — | 1.0[b] | — | 1.4 |
| L-Aspartate | 1.1 | 1.2 | 1.4 | — |
| L-Ascorbate | 1.1 | 1.3 | 1.2 | — |
| Glyoxylate | 1.1 | 0.9 | 0.3 | — |
| β-Phenyl-pyruvate | 1.1 | 0.7 | 0.4 | — |
| Pyruvate, ethyl ester | 0.6 | 0.8 | 0.7 | — |
| Succinate | — | 0.6[b] | — | 1.2 |
| Oxalacetate | — | 0.3[b] | — | 0.4 |
| Malonate | 0.4 | 0.8 | 0.8 | — |

[a] Data included in this table were obtained from several experiments. Different preparations gave some variation in control values; under these conditions, approximately 0.2 to 0.5 mμmoles of acetate are incorporated into fatty acids per 20 min per mg of high-speed supernatant protein (*see also* Table 1). Nevertheless, the response of each preparation to citrate and α-glycerophosphate was uniform. There fore, for purposes of this table, control values are arbitrarily shown as 1.0 to allow comparison of effects of various organic additions from different experiments.

Each sample contained, in a final volume of 1 ml, the following: dialyzed cell-free extract containing 2.9 to 6.5 mg of protein, 70 μmoles of potassium phosphate buffer (pH 6.3), 2.4 μmoles of NADPH₂, 0.05 μmoles of coenzyme-A, 2 μmoles of creatine phosphate, 4.5 units of creatine kinase, 2 μmoles of ATP, 2 μmoles of acetate-*1*-¹⁴C (2 × 10⁶ counts per min per μmole), 60 μmoles of KHCO₃, and the substance to be tested. Samples were incubated for 20 min at 30 C.

[b] For these samples, a crude homogenate containing high-speed supernatant fluid plus the crude ribosomal fraction was used. The remaining samples contained high-speed supernatant.

somal fraction is of interest. It had been previously implied (20) that the crude ribosomal preparation, in contrast to the high-speed supernatant fluid, might not be activated by these

TABLE 3. *Effect of manganese and magnesium on fatty acid synthesis from acetyl-1-$^{14}$C-CoA*

| Metal added[a] | Acetyl-1-$C^{14}$-CoA incorporated into fatty acids (mμmoles) |
|---|---|
| None................... | 2.3 |
| Manganese chloride | |
| 1 × $10^{-3}$ M........... | 26.6 |
| 3 × $10^{-3}$ M........... | 24.0 |
| 6 × $10^{-3}$ M........... | 19.3 |
| 10 × $10^{-3}$ M........... | 10.9 |
| 20 × $10^{-3}$ M........... | 12.6 |
| 30 × $10^{-3}$ M........... | 11.5 |
| Magnesium chloride | |
| 2 × $10^{-3}$ M........... | 5.6 |
| 3 × $10^{-3}$ M........... | 12.8 |
| 10 × $10^{-3}$ M........... | 50.4 |
| 20 × $10^{-3}$ M........... | 57.2 |
| 30 × $10^{-3}$ M........... | 66.8 |

[a] Each sample contained, in a final volume of 1 ml, the following: dialyzed supernatant protein containing 5.7 mg of protein, 70 μmoles of potassium phosphate buffer (pH 7.0), 2.4 μmoles of NADPH$_2$, 2 μmoles of acetyl-1-$^{14}$C-coenzyme-A ($10^6$ counts per min per μmole), 10 μmoles of creatine phosphate, 4.5 units of creatine kinase, 5 μmoles of ATP, and 60 μmoles of KHCO$_3$. Samples were incubated for 20 min at 30 C.

TABLE 4. *Effect of manganese and magnesium on fatty acid synthesis from malonyl-1,3-$^{14}$C-CoA*

| Addition[a] | Malonyl-1,3-$C^{14}$-CoA incorporated into fatty acids (mμmole) |
|---|---|
| None................... | 0.28 |
| Manganese chloride | |
| 2.5 × $10^{-3}$ M........ | 0.20 |
| 5 × $10^{-3}$ M........ | 0.20 |
| 10 × $10^{-3}$ M........ | 0.15 |
| 30 × $10^{-3}$ M........ | 0.05 |
| Magnesium chloride | |
| 2.5 × $10^{-3}$ M........ | 0.28 |
| 5 × $10^{-3}$ M........ | 0.25 |
| 10 × $10^{-3}$ M........ | 0.24 |
| 30 × $10^{-3}$ M........ | 0.24 |

[a] Each sample contained, in a final volume of 1 ml, the following: light particle preparation containing 0.054 mg of protein, 100 μmoles of potassium phosphate buffer (pH 6.3), 1 μmole of NADPH$_2$ 0.1 μmole of acetyl-coenzyme A, 3.5 μmoles of malonyl-1,3-$^{14}$C-CoA (14.3 × $10^3$ counts per min per mμmole). Samples were incubated for 20 min at 30 C.

compounds, since it was suggested that the particulate fraction might contain carboxylase which was already activated. This contention was based on the observations of Vagelos et al. (18) and Matsuhashi et al. (15) that activation of animal carboxylases results in the trimerization of the inactive protein. However, the results obtained here show that the crude ribosomal fraction is subject to stimulation by α-glycerophosphate and citrate of the same order as that found with the high-speed supernatant. This finding suggests that activation of the yeast carboxylase, unlike that of the animal systems, may not involve a change in molecular weight. Indeed, preliminary studies with this enzyme (17) show no change in sedimentation behavior when the enzyme is present with activators in density gradients.

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